INDUCTION OF MOLTING IN THE CRAYFISH, CAMBARUS, BY MODIFICATION OF DAILY PHOTOPERIOD[1]

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Several investigators have been concerned with the modifications in reproductive phenomena in the Crustacea which are induced by subjecting the animals to various daily light rations. Thus Stephens (1952) studied the modifications in oöcyte maturation in the crayfish, *Cambarus,* induced by exposure to various daily photoperiods and postulated a definite role of light in the control of normal reproductive cycles in these animals. Paris and Jenner (1952) extended the observation of photoperiodism in the Crustacea to the fresh water prawn, *Palaemonetes,* and also demonstrated that administration of the daily light ration in a discontinuous fashion may permit stimulation by daily rations which are not effective when administered continuously. Stephens (1953) provided evidence for an influence of day-length on the rate of development of secondary sex characteristics of the crayfish, *Cambarus.*

Hormonal influences on the crustacean sex cycle were first demonstrated by Panouse (1943, 1944, 1946) for the shrimp, *Leander,* and the accelerating effect of eyestalk removal in ovarian development was subsequently confirmed for *Cambarus* and the fiddler crab, *Uca,* by Brown and Jones (1947, 1948). Carlisle (1953) has recently demonstrated an inhibitory factor controlling sex reversal in *Lysmata.* The presence of a molt-inhibiting principle in the sinus gland-X-organ complex has been verified in a number of laboratories (reviewed in Brown, 1952) although this principle may not always be present (Carlisle, 1954) and Echalier (1954) provides evidence suggesting the occurrence of a molt-accelerating principle in the Y-organ of *Carcinus.*

Finally, Scudamore (1948) presents evidence for a functional interrelation of the processes of egg-laying and molt. In view of the hormonal influences on the molting process, the intimate relation between the molting cycle and the reproductive cycle, and the influence of light as an exteroceptive factor controlling reproduction, the possibility of the modification of molting by treatment with various light periods is of considerable interest.

The results of experiments involving exposure of *Cambarus* to various daily periods of illumination which had been undertaken for other purposes suggested that molt frequency might be influenced by this treatment. However, the numbers of animals involved were small and the results inconclusive so that it was thought desirable to conduct observations on a larger group in order to obtain a more reliable indication of a possible influence on molting.

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MATERIAL AND METHODS

The animals employed in the experiment to be described were *Cambarus virilis* collected in an artificial mud-bottom lake north of Chicago, Illinois. They were obtained in mid-September and maintained in the laboratory until early November in a large tank supplied with running water. During this time the tank was shielded with black cloth so that all of the animals were maintained in constant darkness. The animals were rather small (average carapace length about 2 cm.) and were probably immature yearlings. The oöcytes of the females were small and showed no sign of yolk deposition in contrast to the normal situation in mature *C. virilis* females at this season. Also the gonopods of the males were uniformly juvenile in form (form II) while those of mature males are typically form I (sexually active) at this time (Scudamore, 1948).

Early in November, these animals were divided into three groups and maintained under the following lighting conditions:

Group 1 consisted of one hundred and thirty animals which were placed in a tank shielded with black cloth to provide constant darkness.

Group 2 consisted of one hundred and ten animals subjected to normal day-length for the local latitude and season. This day-length varied from an initial maximum of ten hours and thirteen minutes through a minimum of nine hours and seven minutes on December 21st (data from the American Ephemerix and Nautical Almanac).

Group 3 consisted of sixty-five animals receiving twenty hours of light per day from 2 A.M. to 10 P.M. Lighting was provided by two 25-watt, unfrosted incandescent bulbs suspended from a wooden frame approximately ten inches above the water level. This produced an illumination of from 8 to 50 foot candles at the water surface. All tanks were covered with black cloth to prevent the access of undesired illumination and the lights were controlled by automatic time clocks.

The temperature in each tank was taken daily and the water flow was adjusted so that the temperatures in the three tanks were equal. Each tank was examined daily for dead animals and cast exoskeletons. As animals died they were autopsied and the gastroliths, if any, removed and weighed after drying. For some days preceding molt in crayfishes calcium is stored in the form of gastroliths in the anterior wall of the stomach. Scudamore (1947) has studied this cycle in animals induced to molt by removal of the eyestalks and demonstrates an increase in gastrolith weight as these animals approach the time of molt. Consequently gastroliths were weighed to provide some information concerning the position of the animals in the molt cycle. In order to compensate for variation in size of the animals a gastrolith factor was calculated and the results are recorded in this form in Table I. This gastrolith factor consists of the weight of the gastroliths in milligrams divided by the cube of the carapace length in centimeters. This quotient is multiplied by 100 for convenience.

RESULTS

The animals in the three groups showed clear differences with respect to molt and gastrolith formation, and mortality.

Group 1, which was composed of thirty-seven females and eighty-three males, was maintained in constant darkness. This group showed the lowest mortality and the least tendency toward gastrolith formation. The average temperature in this tank for the experimental period was 15.2° C. Seventeen animals died during the sixty-four-day experimental period which is a mortality of 13%. Only one animal of the seventeen which died showed any trace of gastrolith formation. This was a male which died sixty-three days after the beginning of the experiment and had a gastrolith factor of 24. No molts occurred in this group.

TABLE I

Record of the time of death or molt and the gastrolith factors for animals of group 2 (normal daylength) and group 3 (20-hour light)

Day	Group 2 (normal daylength)		Group 3 (20-hour light)	
	Sex	Gastrolith factor	Sex	Gastrolith factor
4	female	0		
6			male	0
8			male	0
9			male female	0 63
11			male	0
13	male male	0 0	female	0
15	male	5	male female	0 0
16			male male	0 49
17	female male male	0 0 0	female female male	0 trace* 3
19	female male male	0 0 0	female male	0 0
20			female female	131 52
21			female female	8 18
23	male male male	0 0 1	male	0
24	male	2	female	0
27	male male male female	0 trace* 10 6	male	0

* Too small to be isolated and weighed.

TABLE I—*Continued*

Day	Group 2 (normal daylength)		Group 3 (20-hour light)	
	Sex	Gastrolith factor	Sex	Gastrolith factor
29	male	0	male male	0 25
30			female male	0 trace*
32	male	0	male male	0 32
34	male male	0 14	female	0
35			female female	0 56
38	male male	6 21		
42	male	trace*	male male	6 35
44	male male female female	successful molt 0 0 22		
48			male	successful molt
50			female male	successful molt 3
53	male	0		
55			female	0
59			male male male male female	successful molt (post-molt) 38 14 0
61	male	15	male female male	successful molt successful molt 1
64	male	0	male	successful molt

Group 2, a group of thirty females and eighty males, was exposed to normal day-length under the conditions described. The average temperature in this tank during the period of observations was 15.2° C. Thirty-one of these animals died during the experimental period which is a mortality of 28%. Twelve of the animals which died showed some development of gastroliths and one male molted forty-four days after the beginning of the experiment. Thus 40.6% (thirteen of thirty-two) of the animals examined showed some indication of molt or its successful completion. The average gastrolith factor for those animals which exhibited gastroliths at the time of death, sex, and gastrolith factor or record of molt for the animals in this group are recorded in Table I.

Group 3, composed of twenty-five females and forty males, was exposed to twenty hours of light per day from 2 A.M. to 10 P.M. The average temperature in this tank was 15.3° C. Forty of these animals died during the observation period which is a mortality of 62%. Eighteen of the animals which died showed some sign of gastrolith formation and one of them died after having successfully molted. Six animals molted successfully and survived during the course of these observations. Hence 54.4% (twenty-five of forty-six) of the animals observed molted or showed an indication of the initiation of molt. The average gastrolith factor for those animals which exhibited gastroliths at the time of death was 29.5. The time of death, sex, and gastrolith factor or molt record for the animals in this group are listed in Table I.

The difference in behavior between the animals maintained in constant darkness (group 1) and groups 2 and 3 appears significant on inspection. This is verified statistically when one considers the percentage of the animals observed which show an indication of molt in the three groups. The difference between group 1 and groups 2 and 3 is more than twice the standard error of the difference. However, this does not suffice to demonstrate the significance of the difference between group 2 and group 3 (difference is 1.2 sigma). Applying Student's t test after assigning a reasonable gastrolith factor to the successful molts, the probability that groups 2 and 3 are not different is less than 0.01. The factor value of 150 which was assigned to animals which molted is probably somewhat low; higher values would emphasize the difference between the two groups.

Concerning the differences in mortality of the three groups, all the differences are significant (greater than twice the standard error of the differences) provided one includes the animals which showed gastroliths at the time of death. However, after these animals are excluded, the death rate of the animals exposed to twenty hours of light per day is still significantly higher than that of the animals in groups 1 and 2.

DISCUSSION

It can be concluded on the basis of these results that exposure of *Cambarus virilis* to rather low light intensities during the winter months is capable of inducing molt in animals which would fail to molt if maintained in constant darkness. It is interesting to note that apparently rather short day-lengths are competent to produce this effect. Light periods to which the group in normal day-length, group 2, was exposed varied from a maximum of ten hours and thirteen minutes to a minimum of nine hours and seven minutes. This is in contrast to the results obtained by Paris and Jenner (1952) where a light period of 11 hours per day failed to induce ovarian development in *Palaemonetes*. These authors conclude that the response of these animals is in many respects analogous to the flowering response in long-day plants. On the other hand, Stephens (1952) observed modifications of yolk deposition and oöcyte maturation induced by rather short day-lengths although the situation is somewhat complicated by the fact that her initial exposures were longer (twelve hours) and decreased gradually.

In the results presented here, it would seem that the difference between the response of the animals to short days and twenty-hour days is quantitative rather than qualitative. If there is a threshold for this response, it lies below ten hours and thirteen minutes.

It does not seem likely that photoperiodic responses can provide a complete explanation for the timing of the events in the molt cycle but it does appear that they would contribute in this regard. The typical molt cycle in *Cambarus* consists of a variable number of molts in the first year followed by an annual molt of the females and a spring and fall molt of the males in succeeding years (van Deventer, 1937). The transition from darkness or very short daily exposures to light to day-lengths of twelve hours or more when the animals emerge from their burrows in the spring would provide a reasonable stimulus for the spring molt in both sexes. It is tempting to refer to the well-known retiring behavior of berried females as a possible factor in the delayed spring molt of this group which typically occurs two to three weeks later than the male spring molt. It would be possible to construct hypotheses to attempt to explain the rest of the molt cycle on the basis of variations in susceptibility to light as a stimulus or quiescent periods but there is not sufficient information available to limit the number of such schemes. Furthermore, the possibility that this response is characteristic only of immature animals cannot be discarded at present.

It should finally be pointed out that the results presented show a clear influence of day-length on the viability of these animals. There is a significant increase in the mortality of those animals which did not show gastroliths at the time of death as well as a clear increase in total mortality with increasing day-length. The fact that this increase occurs in animals which do not show gastroliths might suggest that the increase in mortality is not causally related to the increased tendency to molt. However, such a conclusion would rest on the statement that no animal which fails to exhibit gastroliths has entered the physiological pre-molt stage; this does not seem justified. However, it is of interest to compare this increase in mortality with the results of destalking reported by Brown (1938) which led him to postulate a hormonal effect on viability in crayfishes.

SUMMARY

1. During the winter months, crayfishes maintained at moderate water temperatures (approximately 15° C.) respond to daily illumination by an increased tendency to molt.

2. The strength of this tendency to molt increases with increasing length of the daily periods of illumination.

3. If there is a threshold below which this response does not occur, it lies below a daily period of illumination of approximately ten and one quarter hours per day.

4. There is an increase in mortality in groups exposed to various daily photoperiods with the highest mortality occurring in animals exposed to the longest light period. This is true of animals which fail to show any sign of the initiation of molt at the time of death.

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